An Investigation of Norpropoxyphene Cyclization in Methanol

AUTHORS

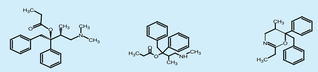
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Abstract

- Dextropropoxyphene, a synthetic opioid analgesic, is widely prescribed for the relief of mild to moderate pain. It is primarily metabolized to norpropoxyphene in humans through N-demethylation. In most methods, analysis of norpropoxyphene involves a strong base-catalyzed conversion to norpropoxyphene amide to help improve the chromatography.[1]
- The solution behavior of norpropoxyphene in the prescence and absence of base was examined by HPLC, LC/MS, and ^{1}H NMR. The results support the formation of a cyclic intermediate in solution. Literature precedence exists for the involvement of a cyclic intermediate in the base-catalyzed conversion of norpropoxyphene to norpropoxyphene amide as well as in the formation of cyclic dinorpropoxyphene.[3,4]
- We propose a mechanism for the formation of this cyclic intermediate and its impact in the analysis of norpropoxyphene by LC/MS. We offer HPLC and ^{1}H NMR evidence which corroborate the formation of this cyclic intermediate in methanol. The effects of storage conditions and time on the formation of this cyclic intermediate in solution will also be discussed.

INTRODUCTION

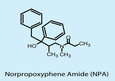
- Dextropropoxyphene is an opioid analgesic structurally related to methadone. It is widely prescribed for the treatment of mild to moderate pain. In addition to the benefits of pain relief, dextropropoxyphene is also used in opioid-dependence therapies for easing the withdrawal symptoms caused by opioid addiction.[1] Recent FDA action of boxed warning requirements for potential overdose highlight the controversial nature of propoxyphene use.[2]
- In humans, dextropropoxypheneis primarily metabolized to norpropoxyphene through N demethylation.[1] Further demethylation and dehydration lead to formation of a second metabolite, cyclic dinorpropoxyphene.[3,4] The presence of dextropropoxyphene and its metabolites in urine or plasma is an indicator of propoxyphene use.



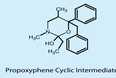
Dextropropoxyphene (DP)

Norpropoxyphene (NP)

Cyclic Dinorpropoxyphene (CDNP)



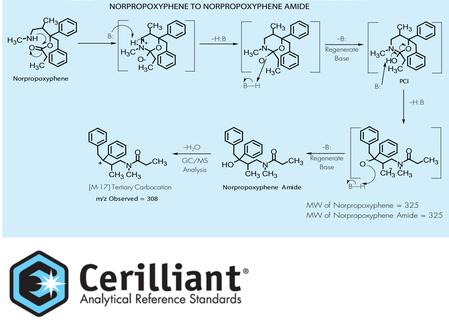
Norpropoxyphene Amide (NPA)



Propoxyphene Cyclic Intermediate (PCI)

- Numerous analytical methods have been published for the quantitative determination of dextropropoxyphene and norpropoxyphene. The most common methods for quantitation of dextropropoxyphene and its metabolites include analysis by GC or GC/MS. In most methods, analysis of norpropoxyphene involves a strong base-catalyzed conversion to norpropoxyphene amide (**NPA**) to help improve the chromatography.[1] Literature precedence exists for the involvement of a cyclic intermediate(**PCI**) in the base-catalyzed conversion of norpropoxyphene to norpropoxyphene amide.[3]
- With the advent of LC/MS and its applications for quantitative determination of dextropropoxyphene and norpropoxyphene, base treatment may not be routinely performed prior to analysis. We were interested in exploring the solution behavior of norpropoxyphene in the absence of strongly basic conditions. The solution behavior of norpropoxyphene in the presence and absence of strong base was examined using HPLC, LC/MS, and NMR.

FIGURE 1. PROPOSED MECHANISM FOR THE BASE CATALYZED CONVERSION OF NORPROPOXYPHENE TO NORPROPOXYPHENE AMIDE



MW of Norpropoxyphene = 325
MW of Norpropoxyphene Amide = 325

OBSERVATIONS FROM NORPROPOXYPHENE ANALYSIS BY GC/MS AND LC/MS

Analysis by GC/MS: Sample treated with base

- Norpropoxyphene maleate neat material or solution treated with base produces m/z 308 [M-17].

Analysis by LC/MS: Without base treatment

- A solution sample of norpropoxyphene maleate prepared in methanol initially shows primary m/z 326 in positive mode and a small amount of an impurity with m/z 308. Over time, the solution sample converts exclusively to the impurity with m/z 308. This peak was designated norpropoxyphene degradant (**NPD**).

The [M-17] ion observed by GC/MS for a sample treated with base is different from the [M-17] ion observed by LC/MS for a sample with no base treatment.

- Why are the [M-17] ions different?
- How are they different?

HPLC, LC/MS, and NMR were used to investigate the differences between analysis of norpropoxyphene by GC/MS and LC/MS involving the presence or absence of base treatment prior to analysis.

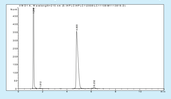
HPLC ANALYSIS OF NORPROPOXYPHENE

Our HPLC analytical method for norpropoxyphene calls for the addition of 0.1 N sodium hydroxide to the standard sample followed by gentle agitation. After allowing the solution to stand for 2 hours at room temperature, the sample is ready for analysis.

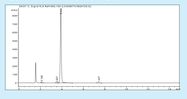
HPLC Method Conditions:

Instrument	Agilent 1100 Series HPLC System
Column	Betasil Phenyl 5µ, 150 x 4.6 mm
Column Temperature	Ambient
Mobile Phases	A: Acetonitrile B: 0.01M KH_2PO_4 buffer
Gradient	Isocratic, 70:30 = A:B
Flow Rate	1 mL/min
Injection Volume	2 µL
Detector	UV/Vis
UV Wavelength	215 nm
Run Time	15 minutes

Base-Catalyzed Conversion of Norpropoxyphene to Norpropoxyphene Amide



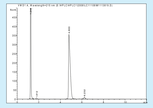
Fresh Sample Of Norpropoxyphene in Methanol



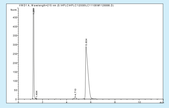
Norpropoxyphene After Base Treatment Step

- The left chromatogram shows an HPLC trace of norpropoxyphene maleate with a retention time of **4.8 minutes.** The right chromatogram represents an HPLC trace of norpropoxyphene amide after base treatment with a retention time of **3.9 minutes**.
- Note conversion to the amide as evidenced by a shift in retention time for the major peak.

Norpropoxyphene Solution Behavior in Methanol in the Absence of Base



Fresh Sample Of Norpropoxyphene in Methanol



Norpropoxyphene in Methanol After Two Weeks at Room Temperature with No Base Treatment (NPD)

Solutions of norpropoxyphene maleate were prepared in methanol at a concentration of 1 mg/mL and monitored by HPLC over 2 weeks at room temperature.

After 2 weeks in methanol at room temperature, a new peak is observed (NPD).

The effects of storage conditions and solvent on the solution behavior of norpropoxyphene in the absence of base were also examined by HPLC.

- Solutions of norpropoxyphene maleate in methanol were stored at room temperature and in the freezer for two weeks. Freezer storage slowed—rather than prevented—the conversion of norpropoxyphene to **NPD**.
- Comparison of norpropoxyphene solution behavior in methanol and acetonitrile indicated that this conversion occurs in both solvents at similar rates.
- Norpropoxyphene from different vendors showed similar behavior in solution.

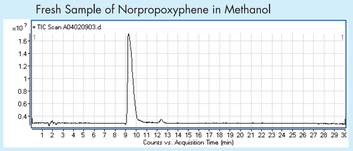
LC/MS ANALYSIS OF NORPROPOXYPHENE

- LC/MS analysis of norpropoxyphene in methanol included comparisons of freshly-prepared and aged samples in the presence and absence of base.
- Different retention times were observed for a base-treated sample of norpropoxyphene, a sample of freshly-dissolved norpropoxyphene in neutral methanol, and a sample of norpropoxyphene in methanol stored in the freezer for 18 months.
- A forced degradation sample of norpropoxyphene (in methanol heated at 60° C for 2 h) had the same retention time as the norpropoxyphene in methanol sample stored in the freezer for 18 months.

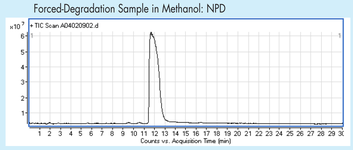
LC/MS Method Conditions:

Instrument	Agilent G6410 Series Triple Quad (QQQ) LC/MS
Column	Betasil Phenyl 5µ, 150 x 4.6 mm
Column Temperature	Ambient
Mobile Phases	A: 5 mM Ammonium formate B: Acetonitrile
Gradient	Isocratic, 40:60 = A:B
Flow Rate	0.8 mL/min
Injection Volume	1 µL
UV Wavelength	215 nm
MS Detector Polarity	Positive
MS Detector	AP-ESI
Mass scan range for full scan	100-800 Da

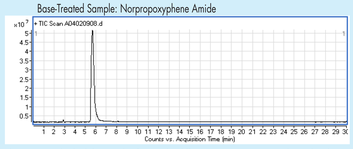
Fresh Sample of Norpropoxyphene in Methanol



Forced-Degradation Sample in Methanol: NPD



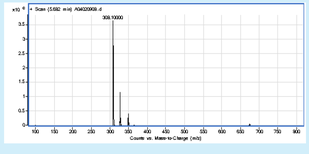
Base-Treated Sample: Norpropoxyphene Amide

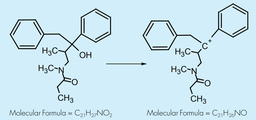


The last two samples both have m/z of 308. The difference in retention times shows that they are not the same.

LC/MS ANALYSIS OF NORPROPOXYPHENE AMIDE

- The presence of norpropoxyphene amide was confirmed in base-treated samples of norpropoxyphene.
- The major ion, m/z 308, corresponds to the tertiary carbocation of norpropoxyphene amide.





Molecular Formula = $C_{21}H_{25}NO_2$

Norpropoxyphene Amide

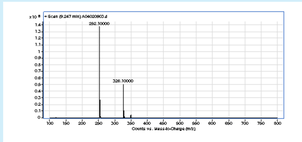
Molecular Formula = $C_{21}H_{25}NO$

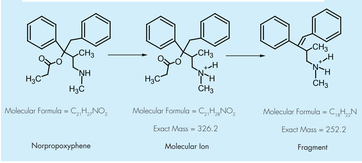
Exact Mass = 308.2

LC/MS ANALYSIS OF NORPROPOXYPHENE SOLUTION BEHAVIOR IN METHANOL

Mass Spectrum of Norpropoxyphene Freshly Dissolved in Methanol in the Absence of Base

- Norpropoxyphene neat material freshly dissolved in methanol was analyzed by LC/MS.
- The presence of norpropoxyphene was confirmed by observation of the parent ion, m/z 326 , and a daughter ion, m/z 252. The parent ion m/z of 326 corresponds to the [M+1] molecular ion of norpropoxyphene.





Molecular Formula = $C_{21}H_{25}NO_2$

Norpropoxyphene

Molecular Formula = $C_{21}H_{24}NO_2$

Exact Mass = 326.2

Molecular Ion

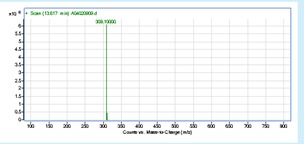
Molecular Formula = $C_{18}H_{21}N$

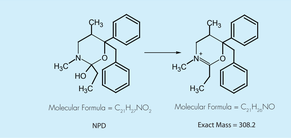
Exact Mass = 252.2

Fragment

Mass Spectrum of NPD in Methanol in the Absence of Base

- An 18 month sample of norpropoxyphene dissolved in neutral methanol was analyzed by LC/MS.
- A single peak with different retention time from norpropoxyphene amide was observed over time in neutral solution (**NPD**). The major ion observed corresponds to a cyclized iminium ion which also has m/z of 308.
- The same retention time and exact mass were observed with the forced-degradation sample.





Molecular Formula = $C_{21}H_{25}NO_2$

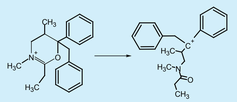
NPD

Molecular Formula = $C_{21}H_{25}NO$

Exact Mass = 308.2

Base Treatment of NPD in Methanol

- LC/MS analysis of **NPD** and norpropoxyphene amide gives the same exact mass for both compounds. Despite the same exact mass, the retention times for both compounds are different on LC/MS.
- When **NPD** is treated with base, it converts to norpropoxyphene amide quantitatively with an observed shift in retention time.



Molecular Formula = $C_{21}H_{25}NO$

Exact Mass = 308.2

NPD

t_R = 13 min

Molecular Formula = $C_{21}H_{25}NO$

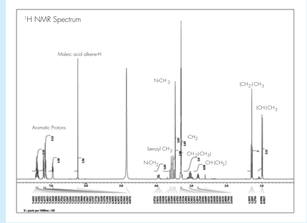
Exact Mass = 308.2

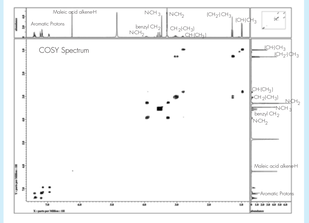
Norpropoxyphene Amide

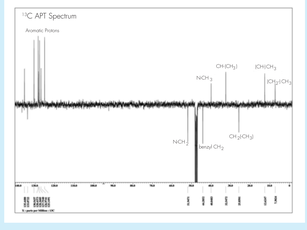
t_R = 5.6 min

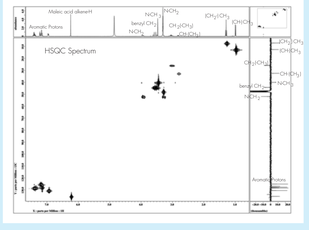
1D & 2D ^{1}H NMR ANALYSIS OF NPD IN METHANOL-D_4

NPD was analyzed by NMR using the following experiments: ^{1}H NMR, COSY, ^{13}C APT, HSQC.

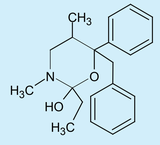








The NMR experiments confirmed the following structure for **NPD**:



Molecular Weight = 325

- The structure of **NPD** is identical to the literature-proposed cyclic intermediate[3] (**PCI**) in the base-catalyzed conversion of norpropoxyphene to norpropoxyphene amide.
- Under LC/MS conditions, the cyclic imminium ion [M-OH] is observed for **NPD**.
- **NPD** quantitatively converts to norpropoxyphene amide with base treatment.

LC/MS and NMR establish that the cyclic structure, NPD, is formed in neutral solution and can be isolated in the absence of base.

STABILITY OF NORPROPOXYPHENE IN SOLUTION

Sample	Norpropoxyphene	NPD	Norpropoxyphene Amide
Neat norpropoxyphene freshly prepared in methanol[A]	97.5%	2.5%	–
Neat norpropoxyphene in acetonitrile after 1 week in the freezer[B]	91.0%	8.9%	–
Neat norpropoxyphene in acetonitrile after 1 week in the refrigerator[B]	84.1%	15.8%	–
Neat norpropoxyphene in acetonitrile after 1 week at room temperature[B]	26.8%	72.4%	–
Neat norpropoxyphene in acetonitrile after 2 weeks at room temperature[B]	4.1%	95.0%	–
Solution standard in methanol, 18 months in sealed ampoule in freezer[A]	0.2%	99.5%	–
Fresh sample heated at 60 °C in methanol for 2 h[A]	1.7%	98.3%	–
Fresh sample in methanol treated with base[A]	–	–	100%
Solution standard in methanol, 18 months in sealed ampoule in freezer, treated with base[A]	–	–	100%

A: LC/MS, % area percent by TIC
B: HPLC, % area percent

- Norpropoxyphene quickly and quantitatively converts to norpropoxyphene amide in basic solution.
- Norpropoxyphene converts over time to the cyclic intermediate **NPD** in neutral solution.
- Nearly complete conversion to **NPD** occurs within 2 hours at 60°C or within 18 months in the freezer.
- **NPD** quickly and quantitatively converts to norpropoxyphene amide in basic solution.
- Conversion of norpropoxyphene to **NPD** occurs in both methanol and acetonitrile at a similar rate.

CONCLUSIONS

- The solution behavior of norpropoxyphene in methanol was examined using HPLC, LC/MS and NMR. The results support the formation of a cyclic product (**NPD**) in neutral solution. **NPD** has the same structure as the proposed cyclic intermediate[3] formed during base-catalyzed conversion of norpropoxyphene to norpropoxyphene amide.
- Neutral solutions (standards or stock solutions) of norpropoxyphene used in testing may contain varying amounts of norpropoxyphene and NPD based on age of the solution, storage conditions, concentration, and solvent. Significant levels of **NPD** (~10%) will be observed with solutions stored in the freezer for just one week.
- Analysis of norpropoxyphene by GC/MS conventionally requires treatment of standards and samples with base, resulting in quantitative conversion to norpropoxyphene amide for analysis.
- Analysis by LC/MS should also include base treatment as a critical sample preparation step. By treating solutions of norpropoxyphene with base, both norpropoxyphene and the cyclized NPD are quantitatively converted to norpropoxyphene amide.

Treating a norpropoxyphene sample or solution standard with base ensures accuracy in quantitation by GC/MS or LC/MS.

REFERENCES

1. Anodhara, G.; Bessard, J.; Vincent, H.; Eyssenc, H.; Bessard, G. J Anal. Toxicol. **1996**, 20, 547-548
2. For more information, refer to: http://www.fda.gov/Safety/MedWatch/SafetyInformation/SafetyAlertsforHumanMedicalProducts/ucm170763.htm
3. Fitzek, J.D.; Bennett, I.F.; Bopp, R.J.; Brunson, M.K.; Sullivan, H.R. J. Pharm. Sci. **1973**, 64, 451.
4. Nishiwm, O.; Rye, T. Arch. Toxicol. **1976**, 36, 99.
5. Sullivan, H.R.; Emmerson, J.L.; Marshall, F.J.; Wood, P.G.; McMahon, R.E. Drug Metab. Dispos. **1974**, 2, 0.

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